Today’s game-plan

• Structure of the course and administrative issues
• Overview of the immune system
• Begin: Innate Immunity

Course Components

• Lecture series
• Discussion sections (not required, but highly recommended)
• Reading: Text (Kuby 6th addition), Journal Articles
• Problem sets (posted on web, these are not graded, but are to help reinforce what you learn in class)
• Exams-- two mid-terms, plus final exam

Grading

• Mid-terms: 25% each
• Final 50%

Half of the material final is a cumulative review and half focuses on the last third of the class.
Course Web Site

- www.mcb.berkeley.edu/courses/mcb150
- All lecture notes, problem sets, answers will be posted for viewing or download
- Powerpoint presentations shown in lecture will be posted for your review

Getting Help!!!

- Web site: www.mcb.berkeley.edu/courses/mcb150
- Discussion groups / GSIs
- Office Hours
- After lecture-- very brief questions only

Overview of the Immune system

Microbes: why they are formidable foes.

- Gross anatomy of the immune system
- Cells of the immune system
- Effector mechanisms: how the immune system protects
- Immune recognition of pathogens: innate vs adaptive immunity
- Cytokines and the inflammatory response

No Discussion sections this week.

- Sign up sheets available in class today
- You may attend any or all of the 4 discussion sections.
- Problem set/reading material for discussion will be posted on website

Microbes are ubiquitous in nature, extraordinarily diverse, rapidly evolve to exploit opportunities to infect hosts and to evade their immune systems.

Many pathogens can expand rapidly in the nutrient-rich environment of the host.

Number of Bacteria

- Exponential growth
- 8 hours = 280 trillion bacteria!!!
Some microbes hijack cellular machinery to replicate and spread. Intracellular pathogens include viruses (influenza, HIV) and intracellular bacteria (listeria) and intracellular parasites (malaria, toxoplasma).

Listeria bacteria using the actin cytoskeleton of the host cell to spread from cell to cell. (Portnoy lab)

Toxoplasma parasites (red) and dendritic cells (green) within a mouse lymph node. (Robey lab)

Overview of the Immune system

Microbes: why they are formidable foes.

First some key definitions:

Pathogen: microbe that causes disease

Antigen: material (from a pathogen) that induces an immune response

Innate (natural) immunity: rapid, non specific immune response

Adaptive (acquired) immunity: slower, specific immune response

Leukocytes: blood cells

Lymphocytes: specialized blood cells that mediate adaptive immunity (e.g. T and B cells)

The cells of the immune system spend much of their time in lymphoid organs. They develop (arise) in primary lymphoid organs, and they interact with antigens in secondary lymphoid organs.

Thymus: primary lymphoid organ for T cell development

Bone marrow: primary lymphoid organ for B cell development

Lymph nodes: collect antigens from tissues

Spleen: collects antigens from blood stream
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Gross anatomy of the immune system

Cells of the immune system

Effector mechanisms: how the immune system protects

Immune recognition: innate vs adaptive immunity

Cytokines and the inflammatory response

Blood cells lineages.

Most blood cells act to fight infection.

Innate immunity

Adaptive immunity

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Immune recognition: innate and adaptive

Cytokines and inflammation

Immune Effector Mechanisms:

Cell-mediated immunity:
- Phagocytosis (cellular eating)
- Cytotoxicity (cellular killing)

Humoral immunity:
- Complement: group of serum proteins that can directly kill pathogens.
- Antibodies: (also called immunoglobulin) proteins secreted by B cells that bind directly and specifically to pathogens. Antibodies target pathogens by marking them for destruction by other components of the immune system.

Many different types of blood cells participate in the immune response to microbes:

Innate immune cells: "phagocytes"
macrophage, neutrophils, dendritic cells

Adaptive immune cells: "lymphocytes"
T cells, B cells

Dendritic cells and macrophage: directly kill microbes by phagocytosis and other mechanisms. They also help to activate T cells (connection between innate and adaptive immunity)

NK cells are lymphocytes that have characteristics of innate and adaptive immunity.
Macrophage fight microbes by engulfing and digesting them (phagocytosis or cellular eating).

A macrophage engulfing yeast

Bacteria fight back against phagocytosis

A macrophage attempting to engulf a bacterium *Streptococcus pneumoniae*, that is covered with a slimy coat.

Target cell killing by a cytotoxic (killer) lymphocyte

A cytotoxic T lymphocyte (CTL) killing a cell that has been infected by a virus. NK cells use a similar mechanism to eliminate tumor cells.

Overview of the Immune system

Microbes: why they are formidable foes.
Gross anatomy of the immune system
Cells of the immune system
Effector mechanisms: how the immune system protects
innate and adaptive immunity
Cytokines and inflammation

Comparison of innate and adaptive immune recognition

| Receptors that mediate innate immune recognition: Toll-like receptors (TLR) |
|---------------------------|-----------------|-----------------|
| TLR1                     | TLR4            | TLR5            |
| TLR2                     | TLR3            | TLR6            |

Receptors that mediate adaptive immune recognition: Antibody and the T cell receptor (TCR)
The genes encoding the antigen receptors of T and B cells are assembled by DNA rearrangement as these cells develop. As a result of V(D)J recombination, every B and T cells expresses a unique version of the antigen receptor.

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Effector mechanisms: how the immune system protects
Immune recognition: innate and adaptive

Cytokines and inflammation

Inflammation: a complex series of events induced by tissue damage (described in medical literature in 1500s)

- Redness (rubor)
- Pain (dolor)
- Swelling (tumor)
- Heat (calor)

Inflammation occurs when injured tissues release mediators that promote vasodilation (increased blood flow) and chemotaxis (directed migration) of leukocytes.

Infection can induce inflammation, but even sterile injuries can be sufficient to induce inflammation.
Blood cells (leukocytes) travel from the blood stream into tissues by a process known as **extravasation**.

Blood cells can also be attracted to sites of infection by products produced by pathogens, as well as by chemoattractants made by host (chemokines, inflammatory mediators).

Neutrophils (a type of white blood cell) are attracted to bacterial products. Here they are moving toward a gradient of the bacterial peptide fMLP.

**Why study the immune system?**

**Importance of the immune system in human health**

- Provides model systems for studies of:
  - gene regulation
  - molecular recognition
  - signal transduction
  - etc, etc

- Provides powerful techniques for use in medicine and science

**Diseases associated with immune system dysfunction**

- Autoimmunity (SLE, Arthritis, Myasthenia gravis, Graves disease)
- Immunodeficiency (inherited, acquired)
- Allergy (environment, drugs)
- Cancer (Leukemia, Lymphoma)
Asthma & Allergy

<table>
<thead>
<tr>
<th>IgE-mediated allergic reactions</th>
<th>Symptom</th>
<th>Common allergens</th>
<th>Route of entry</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>Dermatitis herpetiformis</td>
<td>Intestinal (chief or villus tip epithelium)</td>
<td>Local immune response to allergens (vasoactive substances)</td>
<td></td>
</tr>
<tr>
<td>Acute asthma</td>
<td>Intestinal</td>
<td>Intestinal</td>
<td>Local immune response to allergens</td>
<td></td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>Nasal</td>
<td>Intestinal</td>
<td>Local immune response to allergens</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>Intestinal</td>
<td>Intestinal</td>
<td>Local immune response to allergens</td>
<td></td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>Allergic</td>
<td>Intestinal</td>
<td>Local immune response to allergens</td>
<td></td>
</tr>
</tbody>
</table>

Vaccination: THE major success

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cases per 100,000 population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>100</td>
</tr>
<tr>
<td>Polio</td>
<td>50</td>
</tr>
<tr>
<td>Measles</td>
<td>20</td>
</tr>
</tbody>
</table>

Treatting cancer

<table>
<thead>
<tr>
<th>Cancer-specific antibody</th>
<th>Tumor-specific antibody conjugated to toxin</th>
<th>Tumor-specific antibody conjugated to immunotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody binds to the tumor cell</td>
<td>Antibody-enzyme conjugate binds to the tumor cell</td>
<td>Antibody antibody binds to the tumor cell</td>
</tr>
<tr>
<td>Antibody binds to the tumor cell</td>
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<td>Antibody binds to the tumor cell</td>
</tr>
</tbody>
</table>

Tissue Transplantation

<table>
<thead>
<tr>
<th>Tissue transplanted</th>
<th>5 year graft survival</th>
<th>No. of grafts in USA (1990)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>85-90%</td>
<td>9761</td>
</tr>
<tr>
<td>Liver</td>
<td>45-50%</td>
<td>5964</td>
</tr>
<tr>
<td>Heart</td>
<td>70%</td>
<td>2172</td>
</tr>
<tr>
<td>Lung</td>
<td>30-40%</td>
<td>935</td>
</tr>
<tr>
<td>Cornea</td>
<td>&lt;70%</td>
<td>41000</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>60%</td>
<td>NA</td>
</tr>
</tbody>
</table>

Therapeutics

- Vaccination
- Adoptive immunotherapy
- Transplantation

Powerful methods for detecting and quantitating proteins and cells based on the highly specific binding of antibodies (immunoglobulins)

- ELISA: enzyme-linked immunosorbant assay (measures small amounts of hormones, drugs, microbes in body fluids)
- Western blot (detects disease associated proteins)
- Flow cytometry (quantifies various cell types in mixed population-- HIV patients)
Western Blot

• Separate proteins by size using PAGE gel
• Transfer gel to blotting membrane
• Probe membrane with antibody specific for protein of interest
• Detect bound antibody by chemiluminescence

Immunofluorescence

Flow Cytometry: Quantitative Single-cell Immunofluorescence

Innate (natural) immunity

Innate Immunity

**Innate immune effector mechanisms**
- Physical and biochemical barriers (defensins)
- Phagocytosis and reactive oxygen
- Cell autonomous defenses
  - Apoptosis
  - Interferons and PKR

**Innate immune recognition**
- discovery of the Toll-like receptors
- mammalian TLRs and their ligands
- non-TLR recognition of PAMPs

**Connections between adaptive and innate immunity**
Defensins

- Originally isolated from frog skin based on their ability to kill bacteria
- Small polypeptides (<10kDa) secreted at mucosal surfaces
- Direct bacteriocidal properties
- Insertion into biological membranes leading to target cell lysis
- Inhibited by cholesterol (specificity)

Phagocytosis was discovered by Ilya Mechnikov in 1882

In 1882, the Russian scientist Ilya Mechnikov was working in Messina, Italy, studying the larvae of the sea star. When he inserted a thorn into a larva, something weird happened. Mechnikov noticed strange cells gathering at the point of insertion. The cells surrounded the thorn, eating any foreign substances that entered through the ruptured skin. Mechnikov was thrilled. He decided to name these new cells phagocytes from the Greek words meaning “devouring cells.”

### Phagocytes use a variety of methods to destroy ingested microbes.

<table>
<thead>
<tr>
<th>Oxygen-dependent killing</th>
<th>Oxygen-independent killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive oxygen intermediates</td>
<td>Defensins</td>
</tr>
<tr>
<td>$O_2^-$ (superoxide anion)</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>OH$^-$ (hydroxyl radical)</td>
<td>(macrophage only)</td>
</tr>
<tr>
<td>$H_2O_2$ (hydrogen peroxide)</td>
<td>Lysozyme</td>
</tr>
<tr>
<td>CID (hypochlorite anion)</td>
<td>Hydrolytic enzymes</td>
</tr>
<tr>
<td>Reactive nitrogen intermediates</td>
<td>(household bleach)</td>
</tr>
<tr>
<td>NO (nitric oxide)</td>
<td>Others</td>
</tr>
<tr>
<td>NO$_3$ (nitrogen dioxide)</td>
<td>N$_2$O$_3$ (mononitroxylate)</td>
</tr>
<tr>
<td>HNO$_2$ (nitrous acid)</td>
<td></td>
</tr>
</tbody>
</table>

Phagocytosis “cellular eating”

1. Bacterium attaches to membrane
2. Bacterium is ingested, forming phagosome,
3. Phagosome fuses with lysosome.
4. Lysosomal enzymes digest the bacteria.
5. Digested material is released from cell.

Phagocytes: macrophage, neutrophils, dendritic cells
Macrophage fight microbes by phagocytosis and production of toxic molecules

A macrophage produces reactive oxygen species to aid in destruction of the microbe

Reactive oxygen is revealed by the blue dye, NBT

Innate Immunity

Innate immune effector mechanisms
- Physical and biochemical barriers (defensins)
- Phagocytosis and reactive oxygen species

Cell autonomous defenses
- Apoptosis
- Interferons and PKR

Innate immune recognition
- Discovery of the Toll-like receptors
- Mammalian TLRs and their ligands
- Non-TLR recognition of PAMPs

Connections between adaptive and innate immunity

Some microbes hijack cellular machinery to replicate and spread. Intracellular pathogens include viruses (influenza, HIV) and intracellular bacteria (listeria) and intracellular parasites (malaria, toxoplasma).

cell-autonomous defense: cell produces an immune response that acts on itself

Apoptosis: Cellular Suicide

- Nuclear fragmentation
- Proteolysis
- Blebbing
- Death

Remnants undergo phagocytosis

Apoptosis versus Necrosis

- Tidy: contents of cells degraded from within, producing small cellular “blebs”
- Messy: contents of cell released.
- Programmed from inside the cell
- Induced by external insult

Cell death by necrosis is more likely to produce inflammation.
Interferons are cytokines that are produced in response to viral infection. They produce an "anti-viral state" in target cells. Interferons act on the cell that produces them, as well as neighboring cells. Together with dsRNA, they act to trigger the Protein Kinase R (PKR) pathway. This pathway shuts down the protein synthesis machinery of cells, thus preventing viral replication.

Cells can avoid being hijacked by viruses by activating the Protein Kinase R (PKR) pathway. PKR is triggered by dsRNA and interferon. PKR is a kinase that can be activated by dsRNA or interferon. Once activated, PKR phosphorylates eIF2α, which in turn inhibits translation initiation factors, leading to the inhibition of protein synthesis.

### Innate Immunity

Innate immune effector mechanisms:
- Physical and biochemical barriers (defensins)
- Phagocytosis and reactive oxygen
- Cell autonomous defenses
- Apoptosis
- Interferons and PKR

### Innate immune recognition

**Discovery of the Toll-like receptors**
- TLRs and their ligands
- Non-TLR recognition of PAMPs

Connections between adaptive and innate immunity

### Comparison of the adaptive and innate immune responses

<table>
<thead>
<tr>
<th></th>
<th>Innate</th>
<th>Adaptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response time</td>
<td>hours</td>
<td>days</td>
</tr>
<tr>
<td>Response to repeat infection</td>
<td>identical to primary</td>
<td>stronger response upon second exposure</td>
</tr>
</tbody>
</table>

**Receptors that Mediate pathogen recognition**
- Toll-like receptors (TLRs)
  - Limited diversity
  - Fixed in germline
- Pattern recognition receptors

**Ligands**
- Pathogen associated molecular patterns (PAMPs)
- Virtually any component of pathogens

**Receptors**
- Antibodies and T cell antigen receptors (TCR)
  - Unlimited diversity
  - Generated by V(D)J recombination